Research Highlights

Integrating inflammatory and immunosuppressive circuits in the tumor microenvironment: the pivotal roles of TSLP and FAP-α

TSLP1: a new piece in the puzzle of tumor-associated Th2-type inflammation

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Evaluations of: Pedroza-Gonzalez A, Xu K, Wu TC *et al.*: Thymic stromal lymphopoietin fosters human breast tumor growth by promoting type 2 inflammation. *J. Exp. Med.* 208, 479–490 (2011); De Monte L, Reni M, Tassi E *et al.*: Intratumor T helper type 2 cell infiltrate correlates with cancer-associated fibroblast thymic stromal lymphopoietin production and reduced survival in pancreatic cancer. *J. Exp. Med.* 208, 469–478 (2011).

Tumors may usurp certain mediators and signaling pathways used by normal cells to evade or shift immune responses [1]. Unlocking these mechanisms may help the implementation of novel therapeutic approaches in cancer patients. Two independent studies published in the March issue of the *Journal of Experimental Medicine* [1,2] demonstrate that thymic stromal lymphopoietin (TSLP), an IL-7-related cytokine abundant in the tumor microenvironment, instructs dendritic cells (DCs) to shift the balance towards Th2-mediated inflammatory responses that incite and sustain tumor progression.

Although genetic and epigenetic changes are central to cancer development, tumor progression is dependent on ancillary processes provided by cells of the tumor microenvironment that are not necessarily cancerous themselves [3]. These include mesenchymal stromal cells and fibroblasts that synthesize a plethora of chemokines, cytokines and growth factors; endothelial cells and immature pericytes that support aberrant angiogenesis; and hematopoietic cells that support chronic inflammation [3].

Both experimental and epidemiological data indicate that, irrespective of the trigger for cancer development, a 'smoldering' inflammation is associated with most tumors supporting their progression [4,5]. Tumors promote a constant influx of myelomonocytic cells, which elaborate an array of inflammatory mediators that support protumoral functions, such as proliferation and survival of malignant cells, suppression of adaptive immunity, promotion of angiogenesis and stromal remodeling [3]. Interestingly, while Th1-dependent acute inflammation has been largely associated with tumor rejection, Th2-dependent chronic inflammation has been proposed to facilitate tumor growth [6]. Several cytokines, including M-CSF, IL-4, IL-10 and IL-13, that are present in the tumor microenvironment act in concert to shape the myeloid and lymphoid compartments leading to a shift in anti-tumor T-cell responses [6]. In this regard, chronic inflammation is often associated with the presence of type 2-polarized macrophages (M2), which are induced by the type 2 cytokines IL-4 and IL-13 [4,5]. Tumor-associated macrophages are the major inflammatory component of the tumor stroma, and express several M2-associated pro-tumoral functions including wound healing and angiogenesis [4]. In addition, DCs, which are responsible for orchestrating tumor-specific T-cell responses, are present in an immature or tolerogenic phenotype capable of skewing the immune response toward a Treg- or a Th2-mediated profile [6]. In spite of significant advances, the signaling pathways and mechanisms underlying tumor-associated Th2 responses remain poorly understood.

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Certainly, such pronounced phenotypes cannot be attributed to single cytokines, growth factors or signaling pathways, but are instead mediated by immunoregulatory circuits or networks involving different cell types, cytokines and costimulatory molecules. In this regard, we have recently identified a tolerogenic circuit by which galectin-1, an endogenous lectin abundant in tumor microenvironments, can drive the differentiation of IL-27-producing regulatory DCs that in turn promote the expansion of IL-10-producing Tregs [7]. This circuit may contribute to tumor progression by either dampening Th1-mediated tumor immunity or facilitating Th2-mediated chronic inflammation.

Thymic stromal lymphopoietin is an essential cytokine for the initiation and development of allergic inflammation [8]. Exposure of DCs to epithelial-derived TSLP instructs these cells to express high amounts of OX40L and polarize T cells toward a Th2-dominant profile [8]. Pedroza-Gonzalez and colleagues now demonstrate that this 'Th2-polarizing' circuit is indeed active during the progression of breast cancer [1]. The authors found, using *in vitro* and *in vivo* approaches, that TSLP is secreted from breast cancer cells and favors the expansion of OX40L-expressing DCs, which in turn drive the differentiation of IL-13- and TNF-α-secreting Th2 cells [1]. *In vitro* disruption of OX40–OX40L interactions prevented the differentiation of $CD4^+$ Th2 cells without altering the frequency of IL-10-producing $CD4^+$ T cells, suggesting that these polarized DCs are not typically involved in the generation of Tregs. More importantly, *in vivo* blockade of OX40L partially prevented Th2-dependent inflammation and breast cancer progression. Although OX40L is not constitutively expressed by DCs, it can be induced upon CD40 engagement, cytokine signals or Toll-like receptor stimulation [6]. Pedroza-Gonzalez and colleagues found that OX40L expression by DCs is driven by TSLP secreted from breast cancer cells in both primary and metastatic tumors. Blocking TSLP attenuated Th2-mediated inflammatory responses and partially suppressed tumor progression. Thus, TSLP favors breast cancer progression by fueling tumor-associated type 2 inflammation via mechanisms involving expansion of $OX40L^+$ DCs and subsequent induction of a $CD4^+$ Th2 cell population producing IL-13 and TNF-α, but not IL-4 or IL-10.

Similarly, in the same issue of the *Journal of Experimental Medicine*, De Monte and colleagues found that TSLP is not only secreted by tumor cells but is abundantly released by cancer-associated fibroblasts (CAFs) [2]. This observation is particularly interesting given the critical role of CAFs in supporting tumor progression. The authors identified a cross-talk between tumor cells and CAFs, resulting in a TSLP-dependent induction of Th2-type inflammation. This effect was associated with reduced patient survival in pancreatic cancer. The authors demonstrated that CAF-derived TSLP induces upregulation of its specific receptor on myeloid DCs and acquisition of TSLP-dependent 'Th2-polarizing' potential [2]. Thus, selective blockade of TSLP in CAFs may ameliorate the clinical outcome of pancreatic cancer. Collectively, these findings highlight the importance of Th2 polarization in inflammation-induced cancer progression, underscoring a novel inflammatory circuit mediated by TSLP and downstream molecules including OX40L, IL-13 and TNF-α (Figure 1). Targeting different gears of this circuit might help to delineate novel therapeutic targets to halt inflammation-induced tumor progression in metastatic breast and pancreatic cancer.

References

1 Pedroza-Gonzalez A, Xu K, Wu TC *et al.*: Thymic stromal lymphopoietin fosters human breast tumor growth by promoting type 2 inflammation. *J. Exp. Med.* 208, 479–490 (2011).

2 De Monte L, Reni M, Tassi E *et al.*: Intratumor T helper type 2 cell infiltrate correlates with cancer-associated fibroblast thymic stromal lymphopoietin production and reduced survival in pancreatic cancer. *J. Exp. Med.* 208, 469–478 (2011).

3 Hanahan D, Weinberg RA: Hallmarks of cancer: the next generation. *Cell* 144, 646–674 (2011).

4 Mantovani A, Garlanda C, Allavena P: Molecular pathways and targets in cancer-related inflammation. *Ann. Med.* 2, 161–170 (2010).

5 Coussens LM, Pollard JW: Leukocytes in mammary development and cancer. *Cold Spring Harb. Perspect. Biol.* 3(3), pii: a003285 (2011).

6 Rabinovich GA, Gabrilovich D, Sotomayor EM: Immunosuppressive strategies that are mediated by tumor cells. *Annu. Rev. Immunol.* 25, 267–296 (2007).

7 Ilarregui JM, Croci DO, Bianco GA *et al.*: Tolerogenic signals delivered by dendritic cells to T cells through a galectin-1-driven immunoregulatory circuit involving interleukin 27 and interleukin 10. *Nat. Immunol.* 10, 981–991 (2009).

8 Liu YJ, Soumelis V, Watanabe N *et al.*: TSLP: an epithelial cell cytokine that regulates T cell differentiation by conditioning dendritic cell maturation. *Annu. Rev. Immunol.* 25, 193–219 (2007).

Stromal cells are major players in tumor immune escape

Evaluation of: Kraman M, Bambrough PJ, Arnold JN *et al.*: Suppression of anti-tumor immunity by stromal cells expressing fibroblast activation protein-α. *Science* 330, 827–830 (2010).

Understanding the cellular and molecular bases of tumor immune escape is far from being simple and quite often conclusions obtained using experimental models are incomplete due to a trend toward reductionism in the parameters analyzed [1]. Different strategies are employed by tumors to elude immune recognition or thwart immune responses, including tumor-induced impairment of antigen presentation, activation of negative costimulatory signals (e.g., CTLA-4 and PD-L1) and elaboration of immunosuppressive factors by cancerous cells (e.g., IL-10, TGF-β, prostaglandin E2 and galectin-1) [1]. In addition, a network of regulatory cells of hematopoietic origin contribute to this tolerogenic microenvironment, including Tregs (either $FoxP3^+$ or $FoxP3^-$), NKT cells, myeloid suppressor cells and distinct subsets of myeloid and plasmacytoid dendritic cells. However, emerging evidence made it clear that a whole picture of the tumor microenvironment needs to be considered when studying immune escape strategies by analyzing not only the cancer cell itself but also other cellular components of the tumor microenvironment, regardless of their hematopoietic or nonhematopoietic origin. Following this ground; a recent article by Kraman *et al.* appeared in the journal *Science* illuminating a novel immune-suppressive mechanism mediated by mesenchymal stromal cells [2]. Mesenchymal stromal cells are rare cells that have recently gained popularity due to their ability to maintain homeostasis of connective tissues, their function in tissue regeneration [3], and their multifaceted roles in supporting hematopoietic differentiation [4] and immunomodulation [5].

In this report, the authors analyzed the role of mesenchymal stromal cells in tumor growth (lung and pancreatic carcinomas) and evaluated how these cells influence tumor-specific immune response, particularly those mediated by IFN-γ and TNF-α. To investigate these effects, Kraman and colleagues used *in vivo* genetic engineering approaches to eliminate a stromal cell type of mesenchymal origin that expresses fibroblast activation protein (FAP)-α (**Figure 1**) [2]. Interestingly, depletion of FAP^+ stromal cells resulted in dramatic tumor regression when mice were inoculated with an immunogenic Lewis lung carcinoma cell line. Tumor rejection occurred in immunocompetent, but not in immunodeficient hosts unequivocally demonstrating an immune-mediated effect of FAP^+ stroma cells in promoting tumor growth and fostering immunosuppression. The authors went further to elaborate the mechanistic bases of these observations and found that neither priming nor effector function of antigen-specific $CD8^+$ T cells were affected by depletion of FAP^+ stromal cells. However, this treatment substantially altered the levels of intratumoral IFN-γ and TNF-α. Those proinflammatory cytokines were found to be elevated

when mesenchymal stromal cells were depleted resulting in an acute hypoxic death of the entire tumor mass. However, it still remains to be clarified whether mesenchymal stromal cells have a direct role in the production of these soluble factors or whether they can interfere with accessory cellular circuits that control IFN-γ or TNF-α production (i.e., following uptake of tumor dying cells by cells of myeloid origin). Similarly, previous reports demonstrated that cancer-associated stromal cells produce others soluble factors, such as VEGF and PDGF [6], which positively influence tumor progression by promoting aberrant angiogenesis or attracting immunosuppressive 'M2-type' macrophages [7] or Foxp3$^+$ Tregs [1]. Altogether, these results argue in favor of a major role of mesenchymal stromal cells in facilitating tumor progression, highlighting a novel cellular target for immune intervention. Different therapeutic approaches could be envisioned to interfere with protumorigenic signals secreted by stromal cells or to control the recruitment and/or expansion of mesenchymal stromal cells. While anti-TNF-α or anti-IFN-γ treatments have been proposed, these approaches usually suffer from the toxicity and lack of specificity typical of anticytokine treatment. However, an attractive opportunity for immunointervention involves the selective targeting of mesenchymal stromal cells by capitalizing on their particular cell surface phenotype. In this regard, FAP-α appears to be preferentially expressed by this particular cell type; it is suppressed in normal

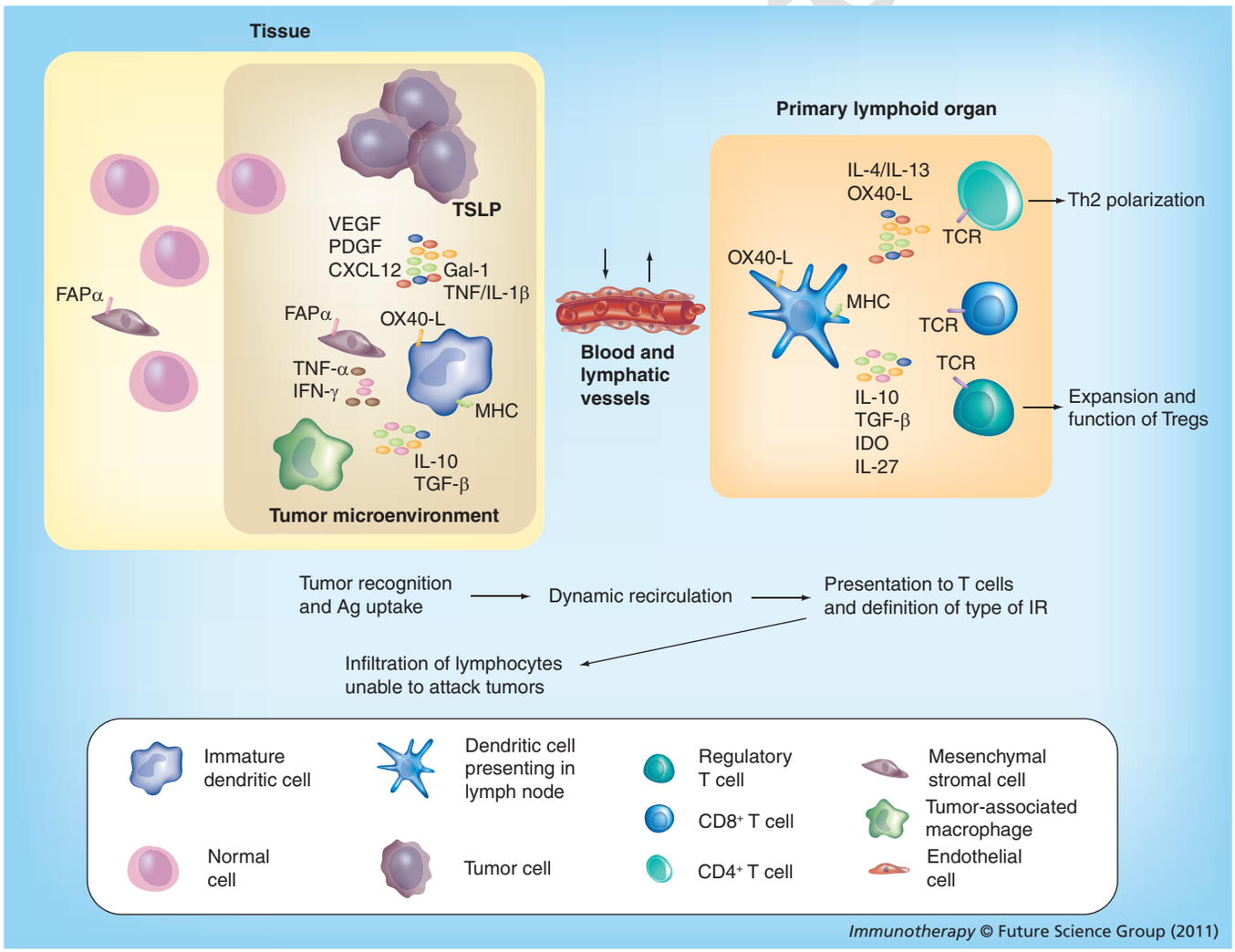

Figure 1. Mechanisms of Th2-dependent chronic inflammation and tumor immune escape that contribute to tumor progression. The figure illustrates the emerging role of the TSLP–OX40L axis in generating Th2-type chronic inflammation mediated by IL-13 and TNF-α and the pivotal role of stromal-derived FPA-α in regulating tumor immune escape.

adult tissues, but is transiently expressed in certain fetal mesenchymal tissues, in reactive stromal fibroblasts associated with epithelial cancers, in fibroblasts supporting wound healing, and in malignant cells of bone and soft-tissue sarcomas [8]. Owing to its scarce distribution in normal tissue and abundant expression in the stroma of over 90% of breast, colorectal and lung carcinomas, blocking FAP-α expression using specific monoclonal antibodies or small-molecules inhibitors may represent a novel therapeutic modality. The current wealth of preclinical and clinical information promises a future scenario in which the synchronized blockade of immunosuppressive mechanisms and deleterious inflammatory mediators may be effective in combination with other conventional strategies to overcome immunological tolerance and Th2-dependent chronic inflammation with the ultimate goal of promoting tumor regression.

References

1 Rabinovich GA, Gabrilovich D, Sotomayor EM: Immunosuppressive strategies that are mediated by tumor cells. *Annu. Rev. Immunol.* 25, 267–296 (2007).

2 Kraman M, Bambrough PJ, Arnold JN *et al.*: Suppression of anti-tumor immunity by stromal cells expressing fibroblast activation protein-α. *Science* 330, 827–830 (2010).

3 Pittenger MF, Mackay AM, Beck SC *et al.*: Multilineage potential of adult human mesenchymal stem cells. *Science* 284, 143–147 (1999).

4 Dazzi F, Ramasamy R, Glennie S, Jones SP, Roberts I: The role of mesenchymal stem cells in haemopoiesis. *Blood Rev.* 20, 161–171 (2006).

5 Le Blanc K, Rasmusson I, Sundberg B *et al.*: Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet* 363, 1439–1441 (2004).

6 Pietras K, Pahler J, Bergers G, Hanahan D: Functions of paracrine PDGF signaling in the proangiogenic tumor stroma revealed by pharmacological targeting. *PLoS Med.* 5(1), e19 (2008).

7 Condeelis J, Pollard JW: Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. *Cell* 124, 263–266 (2006).

8 Dolznig H, Schweifer N, Puri C *et al.*: Characterization of cancer stroma markers: in silico analysis of an mRNA expression database for fibroblast activation protein and endosialin. *Cancer Immun.* 5, 10 (2005).